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(54) Title: STAMEN-SPECIFIC PROMOTERS FROM RICE

(57) Abstract

Rice anther-specific promoters which are of particular utility in the production of transgenic male-sterile monocots and plants for restoring their fertility.

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STAMEN-SPECIFIC PROMOTERS FROM RICE

This invention relates to promoters isolated from rice which can provide gene expression predominantly or specifically in stamen cells of a plant, particularly a monocotyledonous plant, and thereby provide little or no gene expression in other parts of the plant that are not involved in the production of fertile pollen. The promoters are useful in the production of transformed plants, in which a gene is to be expressed at least predominantly, and preferably specifically, in the stamen cells, preferably in the anther cells. The promoters are especially useful in the production of male-sterile plants and male fertility-restorer plants as described in European patent applications ("EPA") 89401194.9 and 90402281.1, respectively (which are incorporated herein by reference), particularly in the production of hybrids of monocotyledonous plants, such as corn, rice or wheat.

Summary of the Invention

In accordance with this invention are provided male flower-specific cDNA sequences isolated from rice comprising the sequences: SEQ ID no. 1, SEQ ID no. 2, SEQ ID no. 3, SEQ ID no. 4 and SEQ ID no. 5 shown in the Sequence Listing. Also in accordance with this invention are provided the stamen-specific, preferably anther-specific, particularly tapetum-specific, promoters of the rice genes corresponding to such cDNA sequences, particularly the promoter PT72 upstream from nucleotide 2846 of SEQ ID no. 6, the promoter PT42 upstream from nucleotide 1809 of SEQ ID no. 7, and the promoter PE1 upstream from nucleotide 2264 of SEQ ID no. 8 shown in the Sequence Listing. These promoters can each be used in a foreign DNA sequence, preferably a foreign chimaeric DNA sequence, which contains a structural gene,

preferably a male-sterility DNA or a male fertility-restorer DNA, under the transcriptional control of one of the promoters and which can be used to transform the nuclear genome of a cell of a plant, particularly a monocotyledonous plant. Further in accordance with this invention are provided: the male-sterile plant or male fertility-restorer plant which can be regenerated from such a cell transformed with the foreign DNA sequence of this invention; the cells, cell cultures and seeds of such a plant; and the male fertility-restored plant and its seeds resulting from crossing such male-sterile and male fertility-restorer plants.

Detailed Description of the Invention

In accordance with this invention, a male-sterile plant or a male fertility-restorer plant can be produced from a single cell of a plant by transforming the plant cell in a known manner to stably insert, into its nuclear genome, the foreign DNA sequence of this invention. The foreign DNA sequence comprises at least one male-sterility DNA or male fertility-restorer DNA that is: under the control of, and fused in frame at its upstream (i.e., 5') end to, one of the stamen-specific, preferably anther-specific, particularly tapetum-specific, promoters of this invention; and fused at its downstream (i.e., 3') end to suitable transcription termination (or regulation) signals, including a polyadenylation signal. Thereby, the RNA and/or protein or polypeptide, encoded by the male-sterility or fertility-restorer DNA is produced or overproduced at least predominantly, preferably exclusively, in stamen cells of the plant. The foreign DNA sequence can also comprise at least one marker DNA that: encodes a RNA and/or protein or polypeptide which, when present at least in a specific tissue or specific cells of the plant, renders the plant easily separable or

distinguishable from other plants which do not contain such RNA and/or protein or polypeptide at least in the specific tissue or specific cells; is under the control of, and is fused at its 5' end to, a second promoter which is capable of directing expression of the marker DNA at least in the specific tissue or specific cells; and is fused at its 3' end to suitable transcription termination signals, including a polyadenylation signal. The marker DNA is preferably in the same genetic locus as the male-sterility or fertility-restorer DNA. This linkage between the male-sterility or fertility-restorer DNA and the marker DNA guarantees, with a high degree of certainty, the joint segregation of both the male-sterility or fertility-restorer DNA and the marker DNA into offspring of the plant regenerated from the transformed plant cell. However, in some cases, such joint segregation is not desirable, and the marker DNA should be in a different genetic locus from the male-sterility or fertility-restorer DNA.

The male-sterility DNA of this invention can be any gene or gene fragment, whose expression product (RNA and/or protein or polypeptide) disturbs significantly the metabolism, functioning and/or development of stamen cells, preferably anther cells, and thus prevents the production of fertile pollen. Preferred male-sterility DNAs are described in EPA 89401194.9, for example those DNAs encoding: RNases such as RNase T1 or barnase; DNases such as endonucleases (e.g., EcoRI); proteases such as papain; enzymes which catalyse the synthesis of phytohormones (e.g. isopentenyl transferase or the gene products of gene 1 and gene 2 of the T-DNA of Agrobacterium; glucanases; lipases; lipid peroxidases; plant cell wall inhibitors; or toxins (e.g., the A-fragment of diphteria toxin or botulin). Other preferred

examples of male-sterility DNAs are antisense DNAs encoding RNAs complementary to genes, the products of which are essential for the normal development of fertile pollen. Further preferred examples of male sterility DNAs encode ribozymes capable of cleaving specifically given target sequences of genes encoding products which are essential for the production of fertile pollen. Still other examples of male-sterility DNAs encode products which can render stamen cells, particularly anther cells -- and not other parts of the plant -- susceptible to specific diseases (e.g. fungi or virus infection) or stress conditions (e.g. herbicides).

The construction of a vector comprising a male-sterility DNA, such as a barnase-encoding DNA, under the control of a rice anther-specific promoter of this invention is most conveniently effected in a bacterial host organism such as E. coli. However, depending on the nature of the male-sterility DNA and the specific configuration of the vector, problems can be encountered due to the expression of the male-sterility DNA in, and the concurrent decrease of viability of, the host organism. Such problems can be solved in a number of ways. For instance, the host organism can be provided, on the same or different plasmid from that containing the male-sterility DNA or even on its chromosomal DNA, with another DNA sequence that prevents or inhibits significantly the effect of the expression of the male-sterility DNA in the host organism. Such an other DNA sequence can encode, for example: an antisense RNA so that the accumulation and translation of the male-sterility RNA is prevented; or a protein (e.g., barstar) which specifically inhibits the gene product of the male-sterility DNA (e.g., barnase; Hartley (1988) J.Mol.Biol. 202, 913). Alternatively, the male-sterility DNA can contain an element, such as a plant intron, which

will only result in an active gene product in a plant cell environment. Examples of introns that can be used for this purpose are introns of: the transcriptional units of the adh-1 gene of maize (Luehrsen and Walbot (1991) Mol. Gen. Genet. 225, 81; Mascarenhas et al (1990) Plant Mol. Biol. 15, 913), the shrunken-1 gene of maize (Vasil et al (1989) Plant Physiol. 91, 1575), the cat-1 gene of castor bean (Tanaka et al (1990) Nucleic Acids Research ("NAR") 18, 6767), and the act-1 gene of rice (McElroy et al (1990) The Plant Cell 2, 163; PCT publication WO 91/09948).

The male fertility-restorer DNA of this invention can be any gene or gene fragment, whose expression product (RNA and/or protein or polypeptide) inactivates, neutralizes, inhibits, blocks, offsets, overcomes or otherwise prevents the specific activity of the product of a male-sterility DNA in stamen cells, particularly in anther cells. Preferred fertility-restorer DNAs are described in EPA 90402281.1, for example those DNAs encoding: barstar which is the inhibitor of barnase; EcoRI methylase which prevents the activity of EcoRI; or protease inhibitors (e.g., the inhibitors of papain). Other examples of fertility-restorer DNAs are antisense DNAs encoding RNAs complementary to male-sterility DNAs. Further examples of fertility-restorer DNAs encode ribozymes capable of cleaving specifically given target sequences encoded by male-sterility DNAs.

The marker DNA of this invention can be any gene or gene fragment encoding an RNA and/or protein or polypeptide that allows plants, expressing the marker DNA, to be easily distinguished and separated from plants not expressing the marker DNA. Examples of the marker DNA are described in EPA 89401194.9, such as marker DNAs

which encode proteins or polypeptides that: provide a distinguishable color to plant cells, such as the A1 gene encoding dihydroquercetin-4-reductase (Meyer et al (1987) Nature 330, 677-678) and the glucuronidase gene (Jefferson et al (1988) Proc. Natl. Acad. Sci. USA ("PNAS") 83, 8447); provide a specific morphological characteristic to a plant such as dwarf growth or a different shape of the leaves; confer on a plant stress tolerance, such as is provided by the gene encoding superoxide dismutase as described in EPA 88402222.9; confer disease or pest resistance on a plant, such as is provided by a gene encoding a Bacillus thuringiensis endotoxin conferring insect resistance on a plant, as described in EPA 86300291.1; or confer on a plant a bacterial resistance, such as is provided by the bacterial peptide described in EPA 88401673.4. Preferred marker DNAs encode proteins of polypeptides inhibiting or neutralizing the activity of herbicides such as: the sfr gene and the sfrv gene encoding enzymes conferring resistance to glutamine synthetase inhibitors such as Bialaphos and phosphinotricine as described in EPA 87400544.0.

In order for the protein or polypeptide encoded by the marker DNA to function as intended, it is often preferred to have it produced in the plant cell as a precursor, in which the mature protein is linked at its N-terminal end to another polypeptide (a "targeting peptide") which will translocate the mature protein to a specific compartment such as the chloroplasts, the mitochondria, or the endoplasmic reticulum. Such targeting peptides and DNA sequences coding for them (the "targeting sequences") are well known. For example, if a marker DNA codes for a protein that confers tolerance or resistance to a herbicide or another selective agent that acts on chloroplast metabolism, such as the sfr (or bar)

gene or the sfrv gene (European patent publication ["EP"] 0,242,236), it may be preferred that such gene also comprise a chloroplast targeting sequence such as that coding for the transit peptide of the small subunit of the enzyme 1,5-ribulose bisphosphate carboxylase (Krebbers et al (1988) Plant Mol. Biol. 11, 745; EPA 85402596.2), although other targeting sequences coding for other transit peptides, such as those listed by Von Heijne et al (1991) Plant Mol. Biol. Reporter 9, 104, can be used.

The stamen-specific, preferably anther-specific, promoters of this invention, such as the promoter PT72 upstream from nucleotide 2846 of SEQ ID no. 6, the promoter PT42 upstream from nucleotide 1809 of SEQ ID no. 7, and the promoter PE1 upstream from nucleotide 2264 of SEQ ID no. 8 -- which can be used to control the male-sterility DNA or the fertility-restorer DNA -- can be identified and isolated in a well known manner as described in EPA 89401194.9. In this regard, each of the cDNAs of SEQ ID nos. 1 to 5 of this invention can be used as a probe to identify (i.e., to hybridize to) the corresponding region of the rice genome (i.e., the region containing DNA coding for the stamen-specific mRNA, from which the cDNA was made). Then, the portion of the plant genome that is upstream (i.e., 5') from the DNA coding for such stamen-specific mRNA and that contains the promoter of this DNA can be identified.

The second promoter, which controls the marker DNA, can also be selected and isolated in a well known manner, for example as described in EPA 89401194.9, so that the marker DNA is expressed either selectively in one or more specific tissues or cells or constitutively in the entire plant, as desired, depending on the nature of the RNA and/or protein or polypeptide encoded by the marker DNA.

In the foreign DNA sequence of this invention, 3' transcription termination signals or the "3' end" can be selected from among those which are capable of providing correct transcription termination and/or polyadenylation of mRNA in plant cells. The transcription termination signals can be the natural ones of the male-sterility or fertility-restorer DNA, to be transcribed, or can be foreign or heterologous. Examples of heterologous 3' transcription termination signals are those of the octopine synthase gene (Gielen et al (1984) EMBO J. 3, 835-845) and of the T-DNA gene 7 (Velten and Schell (1985) NAR 13, 6981-6998). When the foreign DNA sequence of this invention comprises more than one structural gene (e.g., a male-sterility or fertility-restorer DNA and a marker DNA), it is preferred that the 3' ends of the structural genes be different.

In plants, especially in monocotyledonous plants, particularly cereals such as rice, corn and wheat, the expression in accordance with this invention of a marker DNA, as well as a male-sterility DNA or a fertility-restorer DNA, can be enhanced by the presence at one or more, preferably one, appropriate position(s) in the transcriptional unit of each foreign DNA sequence of this invention of a suitable plant intron (Luehrsen and Walbot (1991) Mol. Gen. Genet. 225, 81; Mascarenhas et al (1990) Plant Mol. Biol. 15, 913; Vasil et al (1989) Plant Physiol. 91, 1575; Tanaka et al (1990) NAR 18, 6767; McElroy et al (1990) The Plant Cell 2, 163; PCT publication WO 91/09948). Preferably, each intron has a nucleotide sequence that: is recognizable by the cells of the plant species being transformed (for requirements of intron recognition by plants, see Goodall and Filipowicz (1989) Cell 58, 473; Hanley and Schuler (1988) NAR 16, 7159), is longer than about 70-73 bp (Goodall and Filipowicz (1990) Plant Mol. Biol. 14, 727), and is

positioned close to the 5' end of the encoded mRNA, particularly in any untranslated leader sequence.

Cells of a plant can be transformed with the foreign DNA sequence of this invention in a conventional manner. Where the plant to be transformed is susceptible to Agrobacterium infection, it is preferred to use a vector, containing the foreign DNA sequence, which is a disarmed Ti-plasmid. The transformation can be carried out using procedures described, for example, in EP 0,116,718 and EP 0,270,822 and Gould et al (1991) Plant Physiology 95, 426-434. Preferred Ti-plasmid vectors contain the foreign DNA sequence between the border sequences or at least located upstream of the right border sequence. Of course, other types of vectors can be used for transforming the plant cell, using procedures such as direct gene transfer (as described for example in EP 0,223,247), pollen mediated transformation (as described for example in EP 0,270,356, PCT publication WO 85/01856 and EP 0,275,069), in vitro protoplast transformation (as described for example in US patent 4,684,611), plant virus-mediated transformation (as described for example in EP 0,067,553 and US patent 4,407,956) and liposome-mediated transformation (as described for example in US patent 4,536,475).

Where the plant to be transformed is rice, recently developed transformation methods can be used such as the methods described for certain lines of rice by Christou et al (1991) Bio/Technology 9, 957, Lee et al (1991) PNAS 88, 6389, Shimamoto et al (1990) Nature 338, 274 and Datta et al (1990) Bio/Technology 8, 736.

Where the plant to be transformed is corn, recently developed transformation methods can be used such as the methods described for certain lines of corn by Fromm et al (1990) Bio/Technology 8, 833 and Gordon-Kamm et al (1990) The Plant Cell 2, 603.

Where the plant to be transformed is wheat, a method analogous to those described above for corn or rice can be used. Preferably, for the transformation of a monocotyledonous plant, particularly a cereal such as rice, corn, or wheat, a method of direct DNA transfer, such as a method of biolistic transformation or electroporation, is used. When using such a direct transfer method, it is preferred to minimize the DNA that is transferred so that essentially only the foreign DNA sequence of this invention, with its male-sterility or fertility-restorer DNA and any marker DNA, is integrated into the plant genome. In this regard, when a foreign DNA sequence of this invention is constructed and multiplied on a plasmid in a bacterial host organism, it is preferred that, prior to transformation of a plant with the foreign DNA sequence, plasmid sequences that are required for propagation in the bacterial host organism, such as an origin of replication, an antibiotic resistance gene for selection of the host organism, etc., be separated from the parts of the plasmid that contain the foreign DNA sequence.

The Examples, which follow, describe: the isolation and the characterization of the rice cDNA sequences of SEQ ID nos. 1 to 5 of this invention; their use for isolating, from the rice genome, the stamen-specific promoters of this invention, such as the promoter PT72 upstream from nucleotide 2846 of SEQ ID no. 6, the promoter PT42 upstream from nucleotide 1809 of SEQ ID no. 7, and the promoter PE1 upstream from nucleotide 2264 of SEQ ID no. 8; the construction of gene cassettes by the fusion of each of these promoters with male-sterility and fertility-restorer DNAs; the construction of plant transformation vectors from the promoter cassettes; and the transformation of rice, corn and tobacco with the resulting plant transformation vectors.

Unless stated otherwise in the Examples, all procedures for making and manipulating recombinant DNA were carried out by the standard procedures described in Maniatis et al, Molecular Cloning - A Laboratory Manual, Cold Spring Harbor Laboratory Press, NY (1982), as well as Sambrook et al, Molecular Cloning - A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press, NY (1989). When making plasmid constructions, the orientation and integrity of cloned fragments were checked by means of restriction mapping and/or sequencing.

The sequence identification numbers referred to above and in the Examples are listed below.

Sequence Listing

SEQ ID no. 1 : cDNA sequence of the T72 gene.

SEQ ID no. 2 : partial cDNA sequence of the T23 gene.

SEQ ID no. 3 : cDNA sequence of the T42 gene.

SEQ ID no. 4 : cDNA sequence of the T155 gene.

SEQ ID no. 5 : cDNA sequence of the E1 gene.

SEQ ID no. 6: DNA sequence of rice genomic clone hybridizing to T72 cDNA.

SEQ ID no. 7: DNA sequence of rice genomic clone hybridizing to T42 cDNA.

SEQ ID no. 8: DNA sequence of rice genomic clone hybridizing to E1 cDNA.

SEQ ID no. 9: DNA sequence of plasmid pVE108.

Example 1

Isolation and characterization of anther-specific cDNAs from rice.

For the cloning of cDNAs corresponding to genes which are expressed exclusively, or at least

predominantly, in anthers of rice, a cDNA library was prepared from poly A^+ mRNA isolated from immature spikelets (size 1-3 mm), at their developmental stages of carrying microsporocytes before meiosis and in early meiosis, and from anthers isolated from spikelets (size 3-5 mm), at their developmental stages of carrying microsporocytes undergoing meiosis and after meiosis, from the publicly available rice line Oryza sativa var. japonica, Akihikari. By means of the Amersham cDNA Synthesis System Plus RPN 1256 Y/Z kit (Amersham International PLC, Buckinghamshire, England), cDNAs were synthesized using reverse transcriptase and an oligo dT primer, according to the directions set forth in the kit for its use.

The cDNAs were cloned in lambda gt10 vector, using the Amersham cDNA Cloning System - lambda gt10 - RPN1257 - kit, in accordance to the directions set forth in the kit for its use. Upon the cDNA libraries thus obtained (21,000 plaques for the anther library; 6,000 plaques for the spikelet library), differential screening was performed by hybridization with: a labelled first strand cDNA probe copied from rice immature anther mRNA and a labelled first strand cDNA probe copied from rice immature spikelet mRNA (developmental stages as above) as positive probes; and a labelled first strand cDNA probe copied from rice seedling leaf and a labelled first strand cDNA probe copied from rice seedling root as negative probes. 97 candidate anther- and spikelet-specific cDNA clones were selected and again screened with labeled cDNA probes derived from mRNA of anthers and spikelets of rice (positive probes) and from leaf, root and basis of spikelets of rice (negative probes). The basis of spikelets are immature rice spikelets (size 3-6 mm) from which the anthers and the top of palea and lemma

have been dissected away but which contain intact ovaries. 0.2 µg phage DNA from the 82 candidate clones passing this second selection step was screened for anther-specific expression in a dot blot assay, hybridized with: labelled first strand DNA probes copied from rice immature spikelet mRNA and rice immature anther mRNA (developmental stages as above) as positive probes; and labelled first strand cDNA probe copied from mRNA of rice seedling leaf, rice seedling root, basis of rice spikelet, dry rice seed, rice callus, and axis of immature rice panicle as negative probes (see Table 1). Thus, cDNA clones were identified which hybridize with at least one of the positive probes but for which no hybridization above background was detected with any of the negative probes.

cDNA inserts of 82 candidate clones were purified and hybridized with the collection of 82 candidate clones in order to identify cross-hybridizing and/or overlapping clones. This led to the identification of twenty two anther-specific cDNA clones which show no mutual cross-hybridization and thus are likely to be derived from different genes. Twenty of these clones were shown to correspond to single copy genes in the rice genome (as tested by Southern hybridization; see Table 1) and were subcloned in pGEM2 or pGEM7Zf(+) (PROMEGA, Madison, Wi, USA). 0.2 µg plasmid DNA from the twenty candidate clones was again screened for anther-specific expression in a dot blot assay as described above. Further analysis showed that there were actually only eighteen different inserts, and these inserts were hybridized to Northern blots with 5 µg total RNA from rice immature anther, immature spikelet, leaf and root. It was confirmed that sixteen out of eighteen clones tested are expressed in rice immature anther and immature spikelet (development

stages as above) but not in leaf and root. The profiles of twelve of these selected differential clones, for which a partial or whole sequence was determined, are shown in Tables 1A, 1B and 1C. The twelve cloned anther-specific cDNA inserts were called "T146", "E1", "E2", "T34", "T72", "T157", "T149", "T42", "T139", "T155", "T23", and "T118". Five of these anther-specific cDNAs, i.e., the E1, T72, T42, T155 and T23 cDNAs, were further shown to be expressed both before and after meiosis of microsporocytes and also to exhibit strict anther-specific expression in a more sensitive analysis. The best expression level before meiosis was observed for the E1, T72, and T42 cDNAs. Of these three cDNAs, the T72 cDNA seemed to combine best the desired properties of anther specificity, relatively high level of expression, and substantial premeiotic expression.

The partial or whole sequences of the T72, T23, T42, T155 and E1 cDNAs, cloned in the pGEM plasmids, are shown in SEQ ID no. 1, SEQ ID no. 2, SEQ ID no. 3, SEQ ID no. 4, and SEQ ID no. 5, respectively. The cDNA sequence of T72 reveals two open reading frames (ORF) over 330 and over 114 nucleotides.

Example 2

Isolation of the anther-specific genes corresponding to the anther-specific cDNA clones of Example 1 and identification of their anther-specific promoter regions

To isolate the genomic DNA clones carrying the regulatory sequences of the T72, T23, T42, T155 and E1 genes, corresponding to the selected T72, T23, T42, T155 and E1 cDNAs of Example 1 cloned in the pGEM plasmids pT72, pT23, pT42, pT155 and pE1, respectively, a genomic library of rice var. Akihikari was constructed. This was done by partially digesting Akihikari seedling leaf DNA

with Sau3AI, purifying the 18-22 kb size fraction by a sucrose gradient centrifugation, and cloning in the bacteriophage lambda EMBL3 replacement vector (as described by Frischauff et al (1983) J. Mol. Biol. 170, 827 and in Pouwels et al (1988) Cloning Vectors - A Laboratory Manual (Supplementary Update), Elsevier Science Publishers, Amsterdam) cleaved with BamHI and EcoRI. The library was screened with each of the whole pT72, pT23, pT42, pT155 and pE1 cDNA clones, and the restriction maps of the corresponding genomic clones were determined.

Corresponding genomic clones which hybridize to pT72, pT23, pT42, pT155 or pE1 are sequenced (Maxam and Gilbert (1977) PNAS 74, 560). Comparison of the sequences of pT72, pT23, pT42, pT155 or pE1 and the genomic clones leads to the identification of the homologous regions. For each of the five genes (T72, T23, T42, T155 and E1), the transcription initiation site is determined by primer extension using reverse transcriptase on mRNA of a rice tissue expressing the gene. A "TATA" consensus sequence box is found upstream of the transcription initiation site in the promoter of each of the five genes. The ATG translation initiation site is determined as the most upstream ATG codon in the translational reading frame of each clone, determined by DNA sequencing, and as the first accessible ATG codon on the mRNA synthesized in rice.

DNA sequences of parts of the genomic clones GT72, GT42 and GE1, hybridizing to pT72, pT42 and pE1 respectively, are shown in SEQ ID no. 6, SEQ ID no. 7 and SEQ ID no. 8 respectively. For each sequence, the TATA box and the transcription initiation site is indicated. In each sequence, a reading frame is identified that

starts with an ATG translation initiation codon and that overlaps its corresponding cDNA sequence. The promoter region in each sequence is upstream from, and starts just before, the ATG translation initiation codon of the coding sequence. In this regard, the DNA starting from nucleotide 1 and ending with the nucleotide just before the ATG codon can be considered as the promoter region of each sequence. However, it appears that a preferred portion of each promoter region, for providing anther-specific expression of a heterologous coding sequence of interest (such as a sequence coding for barnase or RNase T1), extends only about 1500 to 1700 bp upstream from its ATG codon, and an even smaller portion of each promoter region extending only about 300 to 500 nucleotides upstream from its ATG codon is sufficient for providing anther-specific expression of a heterologous coding sequence. In each promoter region, the untranslated leader sequence, located between the transcription initiation site and the ATG start of translation, is preferred but is not considered essential for the anther-specific expression of a heterologous coding sequence, and the leader sequence can be replaced by the untranslated leader sequences of other genes, such as plant genes.

A 20 kbp genomic Sau3AI fragment was found that hybridized to the cDNA, pT72. A 4.6 kbp EcoRI fragment of this clone, which hybridized to the cDNA, pT72, was subcloned in pGEM2, and the resulting plasmid was designated "pGT72". A total of 3672 bp, upstream from the EcoRI site closest to the 3' end of the region of homology with the pT72 cDNA, was sequenced, and this sequence is shown in SEQ ID no. 6. By means of primer extension, the initiation of transcription was found to be at position 2765 of this sequence. The TATA box is

presumed to be located between positions 2733 and 2739, while the translation initiation codon is located at position 2846. The sequence upstream of position 2846 can be used as a promoter region, PT72, for the anther-specific, particularly tapetum-specific, expression of a coding sequence of interest. A preferred portion of this promoter region appears to extend from about position 1242 to about position 2845, but the promoter region can comprise the entire sequence between positions 1 and 2845. It also appears that the minimum region which can serve as an anther-specific promoter extends about 300 to 500 bp upstream from position 2846 in SEQ ID no. 6.

Similarly, a genomic Sau3AI fragment (also of about 20 kbp in length) was recovered that hybridized to the cDNA, pT42. A 5 kbp HindIII fragment of this clone, which hybridized to the cDNA, pT42, was subcloned in pGEM2, and the resulting plasmid was designated as "pGT42". A total of 2370 bp, upstream from the HindIII site located within the region of homology with the pT42 cDNA, was sequenced, and this sequence is shown in SEQ ID no. 7. By means of primer extension, the initiation of transcription was found to be at position 1780 of this sequence. The TATA box is presumed to be located between positions 1748 and 1755, while the translation initiation codon is located at position 1809. The sequence upstream of position 1809 can be used as a promoter region, PT42, for the anther-specific, particularly tapetum-specific, expression of a coding sequence of interest. A preferred portion of this promoter region appears to extend from about position 275 to about position 1808, but the promoter region can comprise the entire sequence between positions 1 and 1808. It also appears that the minimum region which can serve as an anther-specific promoter extends about 300 to 500 bp upstream from position 1809 in SEQ ID no. 7.

Similarly, a genomic Sau3AI fragment (also of about 20 kb in length) was recovered that hybridized to the cDNA, pE1. A 6 kbp PvuII fragment of this clone, which hybridized to the cDNA, pE1, was subcloned in the SmaI site of pGEM2, and the resulting plasmid was designated as "pGE1". A total of 2407 bp, upstream from the PvuII site located within the region of homology with the pE1 cDNA, was sequenced, and this sequence is given in SEQ ID no. 8. By means of primer extension, the initiation of transcription was found to be at position 2211 of this sequence. The TATA box is presumed to be located between positions 2181 and 2187, while the translation initiation codon is located at position 2264. The sequence upstream of position 2264 can be used as a promoter region, PE1, for the anther-specific, particularly tapetum-specific, expression of a coding sequence of interest. A preferred portion of this promoter region appears to extend from about position 572 to about position 2263, but the promoter region can comprise the entire sequence between positions 1 and 2263. It appears also that the minimum region which can serve as an anther-specific promoter extends about 300 to 500 bp upstream from position 2264 in SEQ ID no. 8.

Example 3

Construction of promoter cassettes derived from the anther-specific promoter regions of Example 2

The 5' regulatory sequences, including the promoter, of each of the five anther-specific genes of Example 2 are subcloned into the polylinker of pMAC 5-8 (EPA 87402348.4). This produces vectors which can be used to isolate single stranded DNAs for use in site-directed mutagenesis reactions. Using site-directed mutagenesis (EPA 87402348.4), sequences surrounding the ATG translation initiation codon of the 5' regulatory

sequences of each of the anther-specific genes are modified to create a unique recognition site for a restriction enzyme for which there is a corresponding recognition site at the 5' end of each of the male-sterility and fertility-restorer DNAs (that are to be fused to the 5' regulatory sequences in Example 4, below). Each of the resulting plasmids contains the newly created restriction site. The precise nucleotide sequence spanning each newly created restriction site is determined in order to confirm that it only differs from the 5' regulatory sequences of the corresponding rice anther-specific gene by the substitution, creating the new restriction site.

Example 4

Construction of plant transformation vectors from the promoter cassettes of Example 3 and from the anther-specific promoter regions of Example 2

Using the procedures described in EPA 89401194.9 and 90402281.1, the promoter cassettes of Example 3 are used to construct plant transformation vectors comprising foreign chimaeric DNA sequences of this invention, each of which contains the 5' regulatory sequences, including one of the anther-specific promoters, corresponding to each of the five anther-specific genes isolated in Example 2. The 5' regulatory sequences are upstream of, are in the same transcriptional unit as, and control either a male-sterility DNA (from EPA 89401194.9) encoding barnase from Bacillus amyloliquefaciens (Hartley and Rogerson (1972) Preparative Biochemistry 2 (3), 243-250) or a fertility-restorer DNA (from EPA 90402281.1) encoding barstar (Hartley and Rogerson (1972) supra; Hartley and Sweaton (1973) J. Biol. Chem. 248 (16), 5624-5626). Downstream of each male-sterility or fertility-restorer DNA is the 3' end of the nopaline

synthase gene (An et al (1985) EMBO J. 4 (2), 277). Each chimaeric DNA sequence also comprises the 35S3 promoter (Hull and Howell (1987) Virology 86, 482-493) fused in frame with the sfr gene encoding phosphinothricine resistance (EPA 87400544.0) and the 3' end signal of the T-DNA gene 7 (Velten and Schell (1985) NAR 13, 6987).

Example 5

Construction of plant transformation vectors containing the barstar gene under the control of the tapetum-specific promoters of Example 2

Suitable vectors, which carry both the barstar-encoding DNA (Hartley and Rogerson (1972), supra) under the control of the tapetum-specific PT72 promoter of this invention (Example 2) and the herbicide resistance gene, bar (EP 0,242,236), under the control of the 35S3 promoter (EP 0,359,617) and which can be used for the transformation of rice (in Example 7) and corn (in Example 8), are constructed in a procedure comprising four steps as outlined below. Plasmid pVE108, the sequence of which is shown in SEQ ID no. 9, is used.

Step 1.

A DNA fragment, carrying the 3' untranslated end of the nos gene of Agrobacterium T-DNA, is amplified from pVE108 by means of the polymerase chain reaction (PCR; Sambrook et al (1989) supra) using the following two oligonucleotides (CASOL3 and CASOL4) as primers :

CASOL3:

5'-TGG CCA TGG AGG GTA ACC TCC GAA GCA GAT CGT TCA-3'

CASOL4:

5'-CGA ATT CAT ATG CAC GTG TTC CCG ATC TAG TAA CAT-3'.

The resulting fragment is recovered, cleaved with EcoRI and NcoI, and ligated to the large fragment of plasmid

pVE108 cleaved with the same enzymes, yielding plasmid pTSX11.

Step 2.

A fragment containing a tapetum-specific promoter PT72 and a barstar gene is constructed as follows:

1) a DNA fragment, carrying the barstar coding sequence, is amplified from pMT416 (Hartley (1988) J.Mol.Biol. 202, 913) by means of PCR using the following two oligonucleotides (CASOL13T72 and CASOL14) as primers :

CASOL13T72:

5'-CGG CAG AAG ACA CTC ACG GCG ATG AAA AAA GCA GTC ATT AAC-3'

CASOL14:

5'-GGG GGT TAC CTT AAG AAA GTA TGA TGG TGA-3'; and

2) a DNA fragment, carrying the barstar coding sequence under the control of the PT72 promoter of Example 2, is amplified from pT72 (Example 2) by means of PCR using as primers: i) the gel-purified PCR product of step 1), ii) CASOL14, and iii) the following oligonucleotide (T72POL1):

5'-TGG CCA TGG AGC TAG CGG CCG CCA CAG AAC AGG ATA GCA A-3'.

The final fragment contains not only the barstar coding sequence under the control of the PT72 promoter but also comprises: at its 5' end, a linker sequence containing restriction sites for MscI, NcoI, NheI and NotI; and at its 3' end, a linker sequence comprising a BstEII restriction site and a 3 nucleotide spacer (GGG).

Step 3.

The final fragment of Step 2 is recovered, cleaved with NcoI and BstEII, and ligated to the large fragment of

plasmid pTSX11 (Step 1) cleaved with the same enzymes, yielding plasmid pTSX11-T72.

Step 4.

A fragment containing the 35S3 promoter is amplified from pDE9 (EP 0,359,617) by means of PCR using the following two oligonucleotides as primers :

5'-TGG CCA TGG TTA TAG AGA GAG AGA TAG ATT T-3'

5'-GAA GCT AGC AAT CCC ACC AAA ACC TGA ACC T-3'.

The resulting fragment is recovered, cleaved with NcoI and NheI, and ligated to the large fragment of pTSX11-T72 (Step 3) cleaved with the same enzymes, yielding the plasmid designated as "pJVR1-T72".

For constructions with the PE1 promoter instead of the PT72 promoter, the four step procedure, described above, is followed except that in step 2 the following two oligonucleotides (CASOL13E1 and E1POL1) are used instead of CASOL13T72 and T72POL1 respectively:

CASOL13E1:

5'-GAG ATC CAT CAA GCC GTC GCG ATG AAA AAA GCA GTC ATT AAC-3'

E1POL1:

5'-TGG CCA TGG AGC TAG CGG CCG CAG ATC CTT CTG TGT GAT TG-3'.

The plasmid obtained after step 3 is designated as "pTSX11-E1" while the plasmid obtained after Step 4 is designated as "pJVR1-E1".

For constructions with the PT42 promoter instead of the PT72 promoter, the four step procedure, described above, is used except that:

1) in step 2, the following two oligonucleotides (CASOL13T42 and T42POL1) are used instead of CASOL13T72 and T72POL1 respectively:

CASOL13T42:

5'-CAA CTC CCC TCC TCC ACT AGA CCA CCA TGA AAA AAG CAG TCA TTA AC-3'

T42POL1:

5'-GCT AGC GGC CGC ATG GCA GAG CAC GGC CAG-3';

2) the fragment obtained in step 2 is inserted in pTSX11 (Step 3) as follows: the fragment is made blunt end with Klenow and cleaved with BstEII and is then ligated to the large fragment of pTSX11 cleaved with NcoI (filled-in with Klenow) and BstEII. The resulting plasmid is designated as "pTSX11-T42"; and

3) the NotI-HindIII fragment of pJVR1-T72, carrying the bar gene under the control of the 35S3 promoter, is ligated to the large fragment of pTSX11-T42 cleaved with the same enzymes. The plasmid obtained is designated as "pJVR1-T42".

Alternative constructions are also made starting from plasmid pUCNew1 (Example 6). The barstar encoding DNA present on pUCNew1 is first removed by digestion with XhoI and religation, yielding plasmid pUCNew2. The EcoRI-HindIII fragments from pJVR1-T72, pJVR1-E1 and pJVR1-T42, each carrying the barstar-encoding DNA under the control of a rice anther-specific promoter and the bar gene under the control of the 35S3 promoter, are then inserted in the EcoRI and HindIII sites of pUCNew2, yielding pJVR3-T72, pJVR3-E1, pJVR3-T42 respectively.

Plasmids pJVR3-T72, pJVR3-E1, pJVR3-T42, pJVR1-T72, pJVR1-E1, pJVR1-T42 are used for the transformation of rice and corn as described in Examples 7 and 8, respectively.

T-DNA vectors for Agrobacterium-mediated plant transformations are prepared by cloning the appropriate EcoRI (filled-in with Klenow)- HindIII fragments of pJVR1-T72, pJVR1-E1, and pJVR1-T42 (containing the

35S3-bar and rice anther-specific promoter-barnase chimaeric genes) between the HindIII and XbaI (filled-in with Klenow) sites of the known T-DNA vectors pGSC1700 or pGSC1701A. pGSC1700 has been deposited on March 21, 1988 at the Deutsche Sammlung für Mikroorganismen und Zellkulturen (DSM), Mascheroderweg 1B, D-330 Braunschweig, Germany under DSM accession number 4469, and pGSC1701A has been deposited on October 22, 1987 at the DSM under DSM accession number 4286. The T-DNA vectors are used for transformation of tobacco as described in Example 9.

Example 6

Construction of plant transformation vectors containing the barnase gene under the control of the tapetum-specific promoters of Example 2

The tapetum-specific PT72, PT42 and PTE1 promoters of Example 2 are also directly cloned in plant transformation vectors containing the barnase-encoding male-sterility DNA and barstar-encoding fertility-restorer DNA of Example 4. Plasmid pVE108, the sequence of which is shown in SEQ ID no. 9, is used. The plasmid contains a chimaeric gene comprising: the bar gene (EP 0,242,236) under the control of the 35S3 promoter (EP 0,359,617) and with the 3' regulatory sequence of the nopaline synthase gene; and the barnase gene under the control of the tapetum-specific promoter of the TA29 gene (EP 0,344,029) of Nicotiana tabacum and with the 3' regulatory sequence of the nopaline synthase gene. For constitutive expression of the bar gene, an equivalent 35S3 promoter also is used, which differs from the one described in EP 0,359,617 by a 550 bp EcoRI-StuI deletion.

The large NcoI fragment of plasmid pVE108 (filled-in with the large fragment - Klenow - of DNA polymerase I of E. coli) is first ligated to the fragment of the 35S3 promoter as described in EP 0,359,617, amplified by means of the polymerase chain reaction (PCR) using the following two oligonucleotides as primers:

5'-ATT ATA GAG AGA GAG ATA GAT TT-3'

5'-GCA ATC CCA CCA AAA CCT GAA CCT-3'.

The plasmid, in which the NcoI site is reconstructed at the ATG translation initiation codon of the barnase gene, is designated "pVE108del". In this plasmid, the NcoI site at the ATG translation initiation codon of the bar gene is lost.

Then, pVE108del is digested with NcoI, filled in with Klenow, and ligated to one of the following DNA fragments:

1. a 1602 bp fragment obtained by PCR amplification from pGT72 using the following primers:

   5'-ATT CCA CAG AAC AGG ATA GC-3'

   5'-GCC GTG AGT GTC TTC TGC CG-3'.

   The resulting plasmid, in which the promoter fragment from pGT72 is appropriately positioned with respect to the barnase coding sequence, is designated "pVE108-T72";

2. a 1532 bp fragment obtained by PCR amplification from pGT42 using the following primers:

   5'-CCA TGG CAG AGC ACG GCC AG-3'

   5'-GTG GTC TAG TGG AGG AGG GGA GTT G-3'.

   The resulting plasmid, in which the promoter fragment from pGT42 is appropriately positioned with respect to the barnase coding sequence, is designated "pVE108-T42"; and

3. a 1690 bp fragment obtained by PCR amplification from pGE1 using the following primers:

5'-CCT CAG ATC CTT CTG TGT GA-3'

5'-GCG ACG GCT TGA TGG ATC TCT TGC-3'.

The resulting plasmid, in which the promoter fragment from pGT72 is appropriately positioned with respect to the barnase coding sequence, is designated "pVE108-E1".

Alternatively, plasmids pVE108-T72, pVE108-T42 and pVE108-E1 are obtained directly by cloning, in pVE108del, their corresponding promoter fragments obtained by direct PCR amplification from rice genomic DNA using the above-mentioned primers of this Example.

Alternatively, suitable vectors, which carry both the barnase-encoding DNA under the control of the tapetum-specific PT72, PTE1 or PT42 promoter of this invention (Example 2) and the bar gene under the control of the 35S3 promoter and which can be used for transformation of rice (Example 7) and corn (Example 8), are constructed by the four step procedure of Example 5. However, the oligonucleotides used in Step 2 are complementary to the barnase gene in pMT416 instead of to the barstar gene. In this regard, CASOL13T72 is replaced by CASOL15T72, CASOL13T42 is replaced by CASOL15T42, CASOL13E1 is replaced by CASOL15E1, and CASOL14 is replaced by CASOL16. These replacement oligonucleotides are as follows:

CASOL15T72:

5'-CGG CAG AAG ACA CTC ACG GCG ATG GTA CCG GTT ATC AAC ACG-3'

CASOL15T42:

5'-CAA CTC CCC TCC TCC ACT AGA CCA CCA TGG TAC CGG TTA TCA ACA CG-3'

CASOL15E1:

5'-GAG ATC CAT CAA GCC GTC GCG ATG GTA CCG GTT ATC AAC ACG-3'

CASOL16:

5'-GGG GGT TAC CTT ATC TGA TTT TTG TAA AGG TCT G-3'.

The final constructions obtained after step 4 are designated as "pJVR2-T72", "pJVR2-E1" and "pJVR2-T42" respectively.

All vector constructions, containing the barnase-encoding DNA are made in plasmid pMc5-BS in E. coli WK6. Plasmid pMc5-BS contains the barstar-encoding DNA gene under the control of the tac promoter (De Boer et al (1983) PNAS 80, 21) and is constructed by cloning the EcoRI-HindIII fragment of pMT416 (Hartley (1988) J.Mol.Biol. 202, 913) into pMc5-8 (deposited on May 3,1988 at the DSM under DSM accession number 4566). The sequence starting with the PhoA signal sequence and ending with the last nucleotide before the translation initiation codon of the barstar-coding region is deleted by looping-out mutagenesis according to the general procedures described by Sollazi et al (1985) Gene 37, 199. The availability of an ampicillin resistance gene on the pUC18-derived plasmids carrying the chimaeric barnase-coding sequence and the chloramphenicol resistance gene on pMc5-BS permits the strain to be kept stable on plates provided with the two antibiotics or to select for any one plasmid. While normally repressed, gene expression from this promoter can be induced by the addition of a commonly used inducer of the lac operon, IPTG (isopropyl-β-d-thiogalactopyranoside).

Alternatively the barstar-encoding DNA under the control of the tac promoter is inserted in the same plasmid as that carrying the barnase-encoding DNA under

the control of a rice anther-specific promoter of this invention as follows.

In a first step, a new plasmid is constructed by ligation of the three following DNA fragments:

- A DNA fragment, comprising the β-lactamase gene from pUC19 (Yanisch-Perron et al (1985) Gene 33, 103), is amplified from pUC19 by means of PCR using the following two oligonucleotides (CASOL9 and CASOL11):

CASOL 9:

5'-GGA ATT CAA GCT TGA CGT CAG GTG GCA CTT-3'

CASOL11:

5'-TGG GGA GTA AGC TCG AGC CAA AAA GGA TCT TCA CCT AG-3'.

- Another DNA fragment, comprising the origin of replication of pUC19, is amplified from pUC19 by means of PCR using the following two oligonucleotides (CASOL10 and CASOL12):

CASOL10:

5'-GGA ATT CTG ATC AGG CCA ACG CGC GGG GAG A-3'

CASOL12:

5'-TCT TAA TAC GAT CAA TGG CTC GAG TCT CAT GAC CAA AAT CCC TTA-3'.

- Yet another DNA fragment, comprising the barstar-encoding DNA under the control of the tac promoter, is amplified from pMc5-BS by means of PCR using the following two oligonucleotides (CASOL17 and CASOL18):

CASOL17:

5'-CGG CTC GAG CTT ACT CCC CAT-3'

CASOL18:

5'-CCG CTC GAG CCA TTG ATC GTA TTA AGA-3'.

These three DNA fragments are then cleaved with XhoI and EcoRI and ligated to one another. The resulting plasmid, which resembles pUC19 but which has a deleted

lac region, an altered polylinker, and the barstar-encoding DNA under the control of the tac promoter inserted between the β-lactamase gene and the origin of replication of PUC19 (with the barstar-encoding DNA in the same orientation as the β-lactamase gene), is designated as "pUCNew1". The EcoRI-HindIII fragments from pJVR2-T72, pJVR2-E1 and pJVR2-T42, each carrying the barnase-encoding DNA under the control of one of the rice anther-specific promoters of this invention and the bar gene under the control of the 35S3 promoter, are then each inserted in the EcoRI and HindIII sites of pUCNew1, yielding pJVR4-T72, pJVR4-E1 and pJVR4-T42 respectively.

Plasmids pVE108-T72, pVE108-T42, pVE108-E1, pJVR2-T72, pJVR2-T42, pJVR2-E1, pJVR4-T72, pJVR4-T42 and pJVR4-E1 are each used for transformation of rice and corn as described in Examples 7 and 8, respectively.

T-DNA vectors for Agrobacterium-mediated plant transformations are prepared by cloning the appropriate EcoRI (filled-in with Klenow) - XbaI fragments of pVE108-T72, pVE108-T42, pVE108-E1, pJVR2-T72, pJVR2-T42, pJVR2-E1, pJVR4-T72, pJVR4-T42 and pJVR4-E1 (containing the 35S3-bar and rice anther-specific promoter-barnase chimaeric genes) between the HindIII (filled-in with Klenow) and XbaI sites of the known T-DNA vectors, pGSC1700 (DSM 4469) or pGSC1701A (DSM 4286). The T-DNA vectors are used for transformation of tobacco as described in Example 9.

Example 7

Transformation of rice with the plant transformation vectors from Examples 5 and 6

Using the procedures described by Datta et al (1990) supra, protoplasts of the rice line, Oryza sativa var. Chinsurah boro II, are transformed with the plant

transformation vectors described in Examples 5 and 6, and transformed plants are regenerated from the protoplasts.

Alternatively, immature embryos from rice varieties Gulfmont, Lemont, IR26, IR 36, IR54, and IR72 are bombarded with gold particles, carrying appropriate plasmid DNA of Examples 5 and 6, and transformed plants are regenerated from the embryos by the procedures described by Christou et al (1991) Bio/Technology 9, 957. In this regard, transformations with male-sterility DNAs and male fertility-restorer DNAs are carried out using pJVR2-T72, pJVR2-E1, pJVR2-T42, pVE108-T72, pVE108-E1, pVE108-T42, pJVR1-T72, pJVR1-E1, and pJVR1-T42 (Examples 5 and 6), either directly or following suitable linearization after the PT72- and PT42-containing plasmids are digested with EcoRI and HindIII and the PE1-containing plasmids are digested with EcoRI and PstI. These transformations are also carried out with foreign DNA sequences of this invention containing only a male-sterility DNA or a fertility-restorer DNA and a selectable marker DNA, using pJVR4-T72, pJVR4-E1, pJVR4-T42, pJVR3-T72, pJVR3-E1, and pJVR3-T42 (Examples 5 and 6), after being digested with EcoRI and XhoI and then size fractionated by agarose gel electrophoresis or by sucrose gradient centrifugation, so that each foreign DNA sequence can be recovered, digested with XhoI, after which: the fragments are filled-in in a reaction with T4 DNA polymerase, dATP, dCTP, dGTP and biotin-dUTP; and after heat inactivation of the enzymes, the DNA is further digested with EcoRI, and the biotinylated XhoI ends are removed on a streptavidin agarose column (Sigma) or on streptavidin magnetic beads (Promega).

Each transformed plant, containing the tapetum-specific PT72, PT42 or PE1 promoter of Example 2

controlling either a male-sterility DNA or a fertility-restorer DNA, is normal except for its flowers. In this regard, each plant containing a male-sterility DNA under the control of a tapetum-specific promoter expresses such DNA at least predominantly in its tapetum cells and produces no normal pollen, and each plant containing a fertility-restorer DNA under the control of a tapetum-specific promoter expresses such DNA at least predominantly in its tapetum cells but produces normal pollen.

Example 8

Transformation of corn with the plant transformation vectors from Examples 5 and 6

Using the procedures described by Fromm et al (1990) supra,, embryogenic suspension cultures of a B73 X A188 corn line are transformed with the plant transformation vectors described in Examples 5 and 6, and transformed plants are regenerated from the embryogenic suspension cultures. Alternatively, immature embryos from the B73 X A188 corn line are transformed with gold particles carrying the plasmid DNA of Examples 5 and 6, and transformed plants are regenerated from the embryos as described in Example 7. Each transformed plant, containing the tapetum-specific PT72, PT42 or PE1 promoter of Example 2 controlling either a male-sterility DNA or a fertility-restorer DNA, is normal except for its flowers. In this regard, each plant containing a male-sterility DNA under the control of a tapetum-specific promoter expresses such DNA at least predominantly in its tapetum cells and produces no normal pollen, and each plant containing a fertility-restorer DNA under the control of a tapetum-specific promoter expresses such DNA at least predominantly in its tapetum cells but produces normal pollen.

Example 9

Transformation of tobacco with the plant transformation vectors from Examples 4, 5 and 6

Using the procedures described in EPA 89401194.9 and 90402281.1, tobacco plants are transformed by Agrobacterium-mediated transfer with the plant transformation vectors containing the foreign chimaeric DNA sequences from Examples 4, 5 and 6. The transformed tobacco plants, each containing one of the anther-specific promoters of Example 2 controlling either a male-sterility DNA or a fertility-restorer DNA, are normal except for their flowers. In this regard, each plant containing a male-sterility DNA under the control of an anther-specific promoter expresses such DNA at least predominantly in its anthers and produces no normal pollen, and each plant containing a male fertility-restorer DNA under the control of an anther-specific promoter expresses such DNA at least predominantly in its anthers but produces normal pollen.

Needless to say, the use of the anther-specific rice promoters of this invention is not limited to the transformation of any specific plant(s). The rice promoters can be useful in any crop where they are capable of controlling gene expression, and preferably where such expression is to occur at least predominantly, preferably specifically, in stamen cells of the crop. Also, the use of these promoters is not limited to the control of male-sterility DNAs or fertility-restorer DNAs but can be used to control the expression of any gene selectively in stamen cells.

Furthermore, this invention is not limited to the specific stamen-specific, preferably anther-specific, particularly tapetum-specific, promoters described in the

foregoing Examples. Rather, this invention encompasses promoters equivalent to those of Example 2 which can be used to control the expression of a structural gene, such as a male-sterility DNA or a fertility-restorer DNA, selectively in stamen cells, preferably anther cells, particularly tapetum cells, of a plant. Indeed, it is believed that the DNA sequence of each of the promoters of Example 2 can be modified by replacing some of its nucleotides with other nucleotides, provided that such modifications do not alter substantially the ability of polymerase complexes, including transcription activators, of stamen cells, particularly anther cells, to recognize the promoter, as modified.

TABLE 1 : PROFILES OF SELECTED DIFFERENTIAL CLONES

TABLE 1A

name	cDNA size	mRNA size	copy number	dot blot assay for expression in RNA sample		
				anther	spikelet	
					1.5-3 mm	4-6 mm
E1	530	800	1	8	7	7
T72	400	800	1	9	7	9
T157	600	1900	1	7	0	7
T149	500	2600	1	7	2	8
T42	270	800	1	8	7	6
T146	1200		1	4	1	5
T139	200	1200	1	8	6	7
T155	250	900	1	7	4	5
T34	650	800	1	9	8	7
T23	1000	1300	1	8	4	9
T118	700	1100	1	5	7	5
E2	700	800	1	6	6	5

TABLE 1 B

name	cDNA size	mRNA size	copy number	dot blot assay for expression in RNA sample					
				leaf	root	basis of spikelet			
						1	2a	2b	2c
E1	530	800	1	0	0	0	1	1	1
T72	400	800	1	0	0	0	2	1	1
T157	600	1900	1	0	1	0	1	1	1
T149	500	2600	1	0	1	0	1	1	1
T42	270	800	1	0	1	0	2	1	1
T146	1200		1	1	1	1	1	1	1
T139	200	1200	1	1	0	0	2	2	1
T155	250	900	1	1	1	1	1	1	1
T34	650	800	1	2	2	1	2	2	2
T23	1000	1300	1	2	1	2	2	2	2
T118	700	1100	1	3	2	2	2	2	2
E2	700	800	1	3	3	2	2	2	2

TABLE 1 C

name	cDNA size	mRNA size	copy number	dot blot assay for expression in RNA sample		
				dry seed	callus	axis
E1	530	800	1	0	0	0
T72	400	800	1	0	0	0
T157	600	1900	1	0	0	1
T149	500	2600	1	0	0	1
T42	270	800	1	0	0	0
T146	1200		1	0	1	1
T139	200	1200	1	1	1	0
T155	250	900	1	0	1	1
T34	650	800	1	0	2	2
T23	1000	1300	1	0	3	3
T118	700	1100	1	0	3	3
E2	700	800	1	0	4	3

legend : - basis of spikelet subdivision:
1 : "white" spikelets of 6-6.5 mm
2a, 2b, 2c : immature spikelets of 3-5 mm; the three categories correspond to different samples of mRNA from different batches of the same type of tissue
(preparaions of basis of spikelets may have been contaminated with remnants of anthers)
- 1 to 9 : corresponds to expression level ; 0 corresponds to a hybridization level not higher than the background (hybridization obtained with pGEM2 without insert).

- empty boxes : not determined

- mRNA size : has been determined by Northern blot

- copy number : corresponds to the number of hybridizing bands detected with the cDNA inserts as a probe in Southern blots of Akihikari leaf genomic DNA digested with a majority of restriction enzymes tested (AvaI, BamHI, BglII, EcoRI, HindIII, KpnI, MspI, RsaI, and SacI).

SEQUENCE LISTING

1. General Information

i) APPLICANT : PLANT GENETIC SYSTEMS N.V.

ii) TITLE OF INVENTION : Stamen-specific promoters from rice

iii) NUMBER OF SEQUENCES : 9
SEQ. ID. NO 1 : cDNA T72
SEQ. ID. NO 2 : cDNA T23
SEQ. ID. NO 3 : cDNA T42
SEQ. ID. NO 4 : cDNA T155
SEQ. ID. NO 5 : cDNA E1
SEQ. ID. NO 6 : genomic DNA hybridizing to cDNA T72
SEQ. ID. NO 7 : genomic DNA hybridizing to cDNA T42
SEQ. ID. NO 8 : genomic DNA hybridizing to cDNA E1
SEQ. ID. NO 9 : plasmid pVE108

iv) CORRESPONDENCE ADDRESS :
A. ADDRESSEE : Plant Genetic Systems N.V.
B. STREET : Plateaustraat 22,
C. POSTAL CODE AND CITY : 9000 Ghent,
D. COUNTRY : Belgium

v) COMPUTER READABLE FORM :
A. MEDIUM TYPE 5.25 inch, double sided, high density 1.2 Mb floppy disk
B. COMPUTER : IBM PC/AT
C. OPERATING SYSTEM : DOS version 3.3
D. SOFTWARE : WordPerfect 5.1

vi) CURRENT APPLICATION DATA : Not Available

(vii) PRIOR APPLICATION DATA :
EPA 91400318.1, filed February 8, 1991
EPA 91402590.3, filed September 27, 1991
EPA 91403352.7, filed December 10, 1991

2. Sequence Description : SEQ ID NO. 1

SEQUENCE TYPE: nucleotide sequence
SEQUENCE LENGTH: 446 bp

STRANDEDNESS: double stranded
TOPOLOGY: linear
MOLECULAR TYPE: cDNA to mRNA

ORIGINAL SOURCE:
ORGANISM: rice
ORGAN : anther

FEATURES: - Nucleotide (nt) 1 to nt 21 : cloning adaptor sequence
- nt 22 to nt 429 : cDNA T72
- nt 430 to nt 446 : cloning adaptor sequence
Open reading frames starting from start of cDNA sequence
- from nt 22 to nt 144
- from nt 23 to nt 334
- from nt 24 to nt 119

PROPERTIES: cDNA designated as T72

```
CCGGGGATCC GGGTACCATG GCGGCGCTGG GCGCCGTGTC GCACGACTGC      50
GCCTGCGGCA CGCTCGACAT CATCAACAGC CTCCCCGCCA AGTGCGGCCT     100
CCCGCGCGTC ACCTGCCAGT GATGGAGATG GTGTGCCAAG GTAATTGCGT     150
TTGCTCGTGC GAGGATGAGA AGAGAAGATT GAATAAGATG TTTGATGGCA     200
ACAAGTCATC AGGCGATCCG ATCCCTGCAG CTATGAATGG GAGTATACGT     250
AGTAGTGGTC TCGTTAGCAT CTGTGTGTCG CATATGCACG CCGTGCGTGC     300
CGTGTCTGTC CTGCTTGCTC TGCTGATCGT TCAATGAACG ACAAATTAAT     350
CTAACTCTGG AGTGACAAGT CGTTCGAGAT ATACTAATAC TACCATGTGC     400
AGGGTCTTTC AACCAAAAAA AAAAAAAAAC CATGGTACCC GGATCC        446
```

3. Sequence Description : SEQ ID NO. 2

SEQUENCE TYPE: nucleotide sequence
SEQUENCE LENGTH: 347 bp

STRANDEDNESS: double stranded
TOPOLOGY: linear
MOLECULAR TYPE: cDNA to mRNA

ORIGINAL SOURCE:
ORGANISM: rice
ORGAN : anther

FEATURES: - Nucleotide (nt) 1 to nt 332 : cDNA T23
- nt 333 to nt 347 : cloning adaptor sequence

PROPERTIES: part of cDNA designated as T23

```
AGATGGACAC CGCCAGATCA GGGCTCTCGG CTTCCCGCCA TTCCTCTCCG      50
TTCAGCAGAT GTTCGACGAC TCGATCAAGA GCGTCCAGGA CAAGGGCCTC     100
CTTCCTCCTC ATGCTTGATT CATATGATCC ACACAATTAA GCTGCTTGAT     150
TAATTATAAC TAATCAAATA TTGTTAAGGA TCGGAATCAC GTAGTACCGA     200
TCATATATGT GTTCATCTCG AAATTAACTG TAAGTGTGAG ATCGAGAATA     250
CACTAATACA GTGCTAATAT ATACCGAAAT GTTTGTAAAA AAAAAAAAAA     300
AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AACCATGGTA CGGATCC       347
```

4. Sequence Description : SEQ ID NO. 3

SEQUENCE TYPE: nucleotide sequence
SEQUENCE LENGTH: 294 bp

STRANDEDNESS: double stranded
TOPOLOGY: linear
MOLECULAR TYPE: cDNA to mRNA

ORIGINAL SOURCE:
ORGANISM: rice
ORGAN : anther

FEATURES: - Nucleotide (nt) 1 to nt 16 : cloning adaptor sequence
- nt 17 to nt 284 : cDNA T42
- nt 285 to nt 294 : cloning adaptor sequence

PROPERTIES: cDNA designated as T42

```
GAATTCGGTA CCATGGCGCC GCCTGCGGCC TCTCCATCAG CTTCACCATC      50
GCCCCCAACA TGGACTGCAA CCAGGTTACA GAGGAACTGA GAATCTGAGA     100
GCGTGAGGAA TCGAGTTCAT GTTGCATTTA TCATCAATCA TCATCGACTA     150
GATCAATAAA TCGAGCAAAG CTTTGATAAA GAGCGAGCCG CCTTAATTAA     200
TTTACAATAA TCTTGGATGT CATCCTGCAT GYGTGTATGA TCACACGGTT     250
GTTTAATTAG GCACTTTAAT TTTGCAAAAA AAAACCATGG TACC           294
```

5. Sequence Description : SEQ ID NO. 4

SEQUENCE TYPE: nucleotide sequence
SEQUENCE LENGTH: 268 bp

STRANDEDNESS: double stranded
TOPOLOGY: linear
MOLECULAR TYPE: cDNA to mRNA

ORIGINAL SOURCE:
ORGANISM: rice
ORGAN : anther

FEATURES: - Nucleotide (nt) 1 to nt 7 : cloning adaptor sequence
- nt 8 to nt 253 : cDNA T155
- nt 254 to nt 268 : cloning adaptor sequence

PROPERTIES: cDNA designated as T155

```
ACCATGGGTT GTGTTAGCGC GCGGCAAAAG TTACCGTCGT GATCATTTCT      50
GGGCTACTTC CAGCAGGAGA TCGGCCTAGC TGGTGTCTTA ATTAATTATA     100
TGTGATGTGC TGTTCCGTTT TCTGTGATGT GTGTCATCCG TTTCATACTC     150
CGTATCGATC ATCATTATGT GTTTCCGGTA GGAATTTGCG CTCGATATAT     200
GGTGATCCAA AATTTATGAA TCAATTCTTC GTGATTCACT CTGTAAAAAA     250
AAACCATGGT ACCCCGGG                                       268
```

6. Sequence Description : SEQ ID NO. 5

SEQUENCE TYPE: nucleotide sequence
SEQUENCE LENGTH: 617 bp

STRANDEDNESS: double stranded
TOPOLOGY: linear
MOLECULAR TYPE: cDNA to mRNA

ORIGINAL SOURCE:
ORGANISM: rice
ORGAN : anther

FEATURES: - Nucleotide (nt) 1 to nt 58: cloning adaptor sequence
- nt 59 to nt 593 : cDNA E1
- nt 594 to nt 617 : cloning adaptor sequence

PROPERTIES: cDNA designated as E1

```
ACAGGTCGAC TCTAGAGGAT CCCCGGGCGA GCTCGAATTC GAGATCCGGG      50
TACCATGGGC AAGAGATCCA TCAAGCCGTC GCGATGACGA CGAGGCCTTC     100
TGTTTTTTCC ACCGTTGTCG CGGCGATCGC CATCGCCGCG CTGCTGAGCA     150
GCCTCCTCCT CCTGCAGGCT ACCCCGGCCG CGGCCAGCGC GAGGGCCTCG     200
AAGAAGGCTT CGTGCGACCT GATGCAGCTG AGCCCGTGCG TCAGCGCGTT     250
CTCCGGTGTG GGGCAGGGCT CGCCATCGTC CGCGTGCTGC TCCAAGCTCA     300
AGGCGCAGGG CTCCAGCTGC CTGTGCCTCT ACAAGGACGA CCCCAAAGTG     350
AAGCGCATTG TCAGCTCCAA TCGCACCAAG AGGGTCTTCA CCGCGTGCAA     400
GGTGCCCGCG CCGAACTGCT AAGCCTTTGC ATTTGACCAT TGTTCAGTGA     450
GGCAGAAAAC CTGTCACCGC TCGCAGTACT TCTCTCGAGA AAATTAGCAG     500
TAATAAACTC AGTTGAGTGC ATAACAATCT TGGCATGTAC TGTGCATACA     550
GTGTACTTCA AGCTACCCAA ACTCCGAAGC AGTTCTGTCT TCCCCATGGT     600
ACCCGGATCT CGAATTC                                        617
```

7. Sequence Description : SEQ ID NO. 6

SEQUENCE TYPE: nucleotide sequence
SEQUENCE LENGTH: 3627 bp

STRANDEDNESS: double stranded
TOPOLOGY: linear
MOLECULAR TYPE: genomic DNA

ORIGINAL SOURCE:
ORGANISM: Oryza sativa

FEATURES: - nt 1 to nt 2845 : sequence comprising anther-specific PT72 promoter
- nt 2733 to nt 2739 : TATA box
- nt 2765 : transcription initiation determined by primer extension
- nt 2846 : ATG start of translation of T72 gene ; the sequence downstream from this position overlaps with the sequence of the cDNA of SEQ ID No. 1

PROPERTIES: genomic DNA from Oryza sativa designated as GT72

```
GACAATACAT CAAGTAAATC AAACATTACA AATCAGAACC TGTCTAAGAA      50
TCCATCTTAA TTCAGAAAAA AACTCAGATT AGATGTTCAT GCTTCCACCA     100
GAAGCAGGAA TGTGCAACCT ACACTTCCTG TAATTTCCAT ACTACAATGT     150
CCCCACTGAC CACTGTGCCT GATGCTCTAT TAGAATACCA CATCCTCCAT     200
GGCTCCATGT AAATGCATAT AAATTTGACT CTTTAAATTA GTAACTACAA     250
TTTAAAATTT ATCGAACATT GTTCAAATTT ATAAACAGTT TCCCCAAATT     300
TAGATGCTCC CAAATGTACA CAGCTACTAG TAAAGCACCA TCCAGTTTCA     350
CCTGAACAGG ACTGACATAA ATGTGTGAAA AGGGGACGTC ATTCCCCCAA     400
ATACAACTGA ACAATCCTCC ATCAGAACAT TCATTTGATT GACATTACTC     450
GGAGAGATAC AGCTCGCAGG CACACGAGAT TCTTCTGCCT TTCCAATTGC     500
CACGAACCCA CATGTCACAC GACCAACCAA AAAGAGAGAA TTTTTCTTTG     550
CACAAACAAA AAGTGAGATT TTTTTTTCGC CACAAAGGTG CGAACTTTCT     600
TCTCTCTCCC ACTTTCCAAT CAAGAAACGA AGCACTCAAA CCAAGAACAA     650
ACCAAGGAAG GAGAGATCGC TCCCTCTCCC AGAGCAAACG AAAGGAGAGA     700
ACTCAGATGG ATGCGAACTA CTACCTTGCC TCTTTCCCCG GAGAAGCAGC     750
GAAGGAGAAG AGCGCGATGC CGCCGCCGCC GCCGCCTCCG GCAACCTCCG     800
GCTCCGGCGA GTCCGCCTCC TCCTCCTCTC TCACCTCTCT CTTCCCAACC     850
GTGTGGTGTT CGAGAAGCTT TTATGCGAGC GACGTGCAGT GGAAGCGGTT     900
GCTCCCAAGT CAAACTGATG GAGACCACCT ACTATCTTCC TCTTGTTTTC     950
TTCTGCTTTT CTTTTCTTTA TCTTTTTTCT TTCATTTTAT TTTGAGCGAT    1000
GAACTTGAGA ACAGTTTGGT TGTGGGTTAA ATTAAACGGT GCAGAATTGC    1050
AAAGCTACGT CCTTTTCGTC TGATTAAGGT GGTATCAGAA TCCTAATCTG    1100
TTAGCTCAGC ATTTGTTTTT GTGTGTTTAA TTGGCCATGA CATCAGATGG    1150
TTCAGACCGG TGGCAGGTCT TCATCGGAGA GGAGAATGAG AGCAATGCAA    1200
GTTGCAAACA ACAAACAGGT CCTTCCAAAC GGGTTGGTTT CATTCCACAG    1250
AACAGGATAG CAACCAGAGC ACAAACCGTT CAACAATATA TATATATATA    1300
TATATATATA TATATATATA TATATATATA TATATATATG ATTTAAAATT    1350
ATATTACTAT TTTTAGGATA CGGAACTCTT AACACATGAA AATCTAAACA    1400
TTTTCAACCA ATCAGAACTA CTAGAAAGAT AATCTAACTA CTTCAAAATT    1450
```

```
TAAAATTTGA CAAATAAAAT AACTAGTTTT TTCTAAAGCT ATCTTCACTG      1500
GACAACTTAT GAATATTTAT ATTTATGAAG CGAGTACTCT CCTAGTACAT      1550
ATTACATATA TATTCTTCTT CTCATGAAAA ATTAACTTCT CGCTATAAAT      1600
CCGAACATAT ATTATGCGTA GCAAGTTGTT TTTTTTAACG GGTGGAGTAA      1650
TATTAGAGTA TTTAAATTCC TTCAAATTGC CATCCCTCTG GGACTTTGCT      1700
GCTGTTGTTC TTCCACGGTT GCTGTCAGTG TCACCCAGAT TTGCATCCTT      1750
TCCAGCTCGT AGCTACTGTT CTGCATGTAT TGGACTTGGA TTAAGATCAA      1800
ATGCAGTTGC TATTGTAACT GCACAATAGC AACTGCACAC AATCATGTCC      1850
ATTCGTTTTC AGATCCAACG GCTCTAGATG ACTGCTACAG TACATGCATA      1900
ATAGTACATC TCTGCTACAG TGTTTTTGCT GCAGTACCAC TTCATATCCT      1950
GGCCTTCCGT TCTAGATCAT GTGATGTACA TGTTTTTTTG AAACAACCCG      2000
CACAAGACAT TGATAGAGTA GGAAATGTGA TGTACATGTT AACGGCTTAA      2050
GTTACAGTTA CAATAACAAC TGCACAGGAT CTTGATCCAT TGGACTTGTA      2100
TAATATCTCA TCTCGTCGTT CCATTATCGT GGTAACAGTT GGCAACTTGG      2150
CATCCAGTGC TGGAAACTAT GCCGTGTGTA CATCAGGATC GTCCTTTTTG      2200
TTCAGTTCCA AGATAGAACA AGTCCAAAAG ATGGCCGTAG TTTTTTTAGT      2250
CACAGTGGAA GCTGACATAG CCGTGGAATA AGTTCTGCAC AAAAGTTGCC      2300
ATTCGAGATC AACTACTGGT AGTAGTAGTC ATCTTCTACC ACTGCGAATA      2350
TTCGAAGGGA CACAAAAAGA TCAACGAGTA AATTAGTTCA CCGGAAGACG      2400
ACACATTATC ACCACAAAAA GACTAAAAAC AAAAAGAAAT TGCCAGGCCA      2450
AAAAAGGCAA AAAAGAAAAA AAAAGATGGC ACGAGGCCCA GGGCTACGGC      2500
CCATCTTGTC GCCGGCCCAA CCGCGCGCGC GAAACGCTCT CGTCGGCTCT      2550
CGGCTCGCCG CGACGCGATG GAGAGTTCGC GCCGCGGCGC GCGCGCGCGT      2600
TCGGTGGCTC ACACGCTTGC GCCCTCGTCC TCCCGGCCGG CGCGGGCGCC      2650
GACCGCGCGT CCGCCGCATG CGCGCGGCGT AGGTGAGCAA CGCGGGCCTC      2700
GCCGCGCGCG CTCCCCTCCT TCGATCCCCT CCTATAAATC GAGCTCGCGT      2750
CGCGTATCGC CACCACCACC ACGACACACA CGCACGCACC GTGCAGGCAT      2800
CGACGACGAG CGAGAGCCCC TCGGCGGCAG AAGACACTCA CGGCGATGGC      2850
GGTGACGAGG ACGGCGCTGC TGGTGGTGTT GGTAGCGGGG GCGATGACGA      2900
TGACGATGCG CGGGGCGGAG GCGCAGCAGC CGAGCTGCGC GGCGCAGCTC      2950
ACGCAGCTGG CGCCGTGCGC GCGAGTCGGC GTGGCGCCGG CGCCGGGGCA      3000
GCCGCTGCCG GCGCCCCCGG CGGAGTGCTG CTCGGCGCTG GGCGCCGTGT      3050
CGCACGACTG CGCCTGCGGC ACGCTCGACA TCATCAACAG CCTCCCCGCC      3100
AAGTGCGGCC TCCCGCGCGT CACCTGCCGT AAGAAAACGA ATAAAATCGA      3150
TTTGCTATCT ATCGATGATT GTGTTTTTGT AGACTAAACT AAACCCCTAT      3200
TAATAATCAA CTAACCGATG AACTGATCGT TGCAGAGTGA TGGAGATGGT      3250
GTGCCAAGGT AATTGCGTTT GCTCGTGCGA GGATGAGAAG AGAAGATTGA      3300
ATAAGATGTT TGATGGCAAC AAGTCATCAG GCGATCCGAT CCCTGCAGCT      3350
ATGAATGGGA GTATACGTAG TAGTGGTCTC GTTAGCATCT GTGTGTCGCA      3400
TATGCACGCC GTGCGTGCCG TGTCTGTCCT GCTTGCTCTG CTGATCGTTC      3450
AATGAACGAC AAATTAATCT AACTCTGGAG TGACAAGTCG TTCGAGATAT      3500
ACTAATACTA CCATGTGCAG GGTCTTTCAA CCAAGGTTCA TGTTTTCCAC      3550
GAAAGCCGAT TGAAACGAAA CCGCGAAATT TTGATGCGAG ATGAAAGCAG      3600
ATTCCGAGTG AAATTTTAAA TGGTTTT                               3627
```

8. Sequence Description : SEQ ID NO. 7

SEQUENCE TYPE: nucleotide sequence
SEQUENCE LENGTH: 2370 bp

STRANDEDNESS: double stranded
TOPOLOGY: linear
MOLECULAR TYPE: genomic DNA

ORIGINAL SOURCE:
ORGANISM: Oryza sativa

FEATURES: - nt 1 to nt 1808 : sequence comprising anther-specific PT42 promoter
- nt 1748 to nt 1755 : TATA box
- nt 1780 : transcription initiation site determined by primer extension
- nt 1809 : ATG start of translation of T42 gene ; the sequence downstream from this position overlaps with the sequence of the cDNA of SEQ ID No. 3

PROPERTIES: genomic DNA from Oryza sativa designated as GT42

```
GGCCATCACT GTCGGGTGCT GCGCCATGGA CATCACCGTC TCCTTCCTGC      50
GCCGCCGTCG CCGGTGAGCT CCAAGGCCGA AGCCTTCTTC CCCTCACGCC     100
ACTACCTCTC TCTTCCCCAA TTCCGGCCAA CGCCGTCCGT TGCCACAGCG     150
CCACCTCCAC GCCATCCCAG AGCCCCGTGC CGTGCCACCG GGTTCGCCTC     200
CATCTCCTCT TGCCAACGCC GACGCTCGTC GCGGCAGCCA TGCGCTGTCA     250
CCGATGAACA CCGCCGCGCC ACAGCCATGG CAGAGCACGG CCAGGGAGCC     300
ATGGCTGCTC TGCCTCCTCC TCCTTCTCTC ACATCTGGTT GCAGCCGGAC     350
CTAGTCGGCT TATACAAATG GCCCATGGGC AAAATTGTCT TTTATGAAAG     400
TTTCTCTCAC CGTTTCAGTC GGAAATAATA AAATAATGGG AGGATTGTCC     450
GCCAGCAAAT TACCATATTT TTTCGGTGTC CAAGAGCAAA TACACGATCT     500
TCGGGTGTTT CACAGCAAAG ACCACAATTT CTAAGTGTCC TGTAACAAAT     550
TTTGCCAATA AAAATTTAAA ACCAAAGGAG AAGACTGTAC ATGAAGAAAA     600
ACAAAGAGAA TGAAATTACA TAAGCTCAGG GGTTATAAAG TTGATTTATT     650
TTTAGGATGA AGGAAGTGTG TGAAAACAAT GGCCAATTGG GTGTCGGAAA     700
ATATAACGTG CTTGCTAAAA TGTCGTCCCC ATATCCTGTA GCTGATTATA     750
GATAGACCCT GATGGTCAAG ATGCCCTGTA CTGGATCGTG TTTCCATGCT     800
TCATCTCCGC TTCTCTCAAG TACTCCCCGA ACTCACATAT CTGGTGGGCT     850
GGATCCACAG TAAGAAACAG TCAAACAACA CTCACTTCAT AGATAACCAA     900
TTGTTTAATT ATTCTTAGTC CCTTATCTTA TACTCCTAGT AAGTGCTTAA     950
AAACTTGGTA TAAATATCAA ATTTATCGTA CAATTACAAT ATAATTATAA    1000
CGTATACCAT GTAATTTTTA AAACTATTTT TAGATAAAAA AAATATGGTG    1050
ATGAGCAGCC GCAGCAGCGG ACGCCGAACC ACCTGCCGAA CATCACCAAG    1100
ATAGCGAGTC CTAAAAATTT TTAGTGTTCG TTTGCTGGGT TGGTAACTAA    1150
TTAAAAAAAA AGAGCGACTC ATTAGCTCAT AAATAATTAC GTATTAGCTA    1200
ATTTTTTTAA AAAATAAATT AATATAACTT ATAAAGCAGC TTTTGTATAA    1250
TTTTTTTTTT AAAAAAGTGT TGTTTAGCAG TTTTGGGAAG TGTGCCGAGG    1300
GAAAACGATG AGATGGGTTG GGGAAGGAGG GGGAAGAAGT GAAGAACACA    1350
GCAAATATAG GCAGCATCGT CCCGTACAGA TCAGGCTGCA ACCACGCCCC    1400
```

```
GCGGAGATAG TTAACGCGGC CCACGTTGTG CTATAGCCCG TCACTCTCGC      1450
GGGCCTCTCC AACCTCCAGT TTTTTTTCTA GCCCATCAGC TGATACGGGG      1500
CCTTCCCCCC ATGCAGGAGG ATGGCCCGCC ACGCGGTGTT TTGGGCCGTT      1550
CTCGCCGCGC GCGCCCGTGC CGATCCGGGA CTCATCCCAC GTGCCGCCTC      1600
GCCACCGCCG CCGCCGCCGC TGCTGCTCCG GCTGCCGGCT GGACCTTCAC      1650
GCTCACGCGC TCTCCCCTGC CCAACCACCA CGCAAACAAA CACGAAGTTC      1700
GCGCCGTCGA CCGGCTCCCC TCCTCCCCCG CGCGCATCGG ATCCCCCTAC      1750
ATAAACCCTC TCGCTCGCCA TCGCCATGGC AGCAACTCCC CTCCTCCACT      1800
AGACCACCAT GCACAGATCG ATGGCCTCTC AGGCGGTGGC GCCCCTCCTC      1850
CTCATCCTCA TGCTCGCGGC GGCGGCGGGG GGCGCGTCGG CGGCGGTGCA      1900
GTGCGGGCAG GTGATGCAGC TGATGGCGCC GTGCATGCCG TACCTCGCCG      1950
GCGCCCCCGG GATGACGCCC TACGGCATCT GCTGCGACAG CCTCGGCGTG      2000
CTCAACCGGA TGGCCCCGGC CCCCGCCGAC CGCGTCGCCG TCTGCAACTG      2050
CGTCAAGGAC GCCGCCGCCG GCTTCCCCGC CGTCGACTTC TCCCGCGCCT      2100
CCGCCCTCCC CGCCGCCTGC GGCCTCTCCA TCAGCTTCAC CATCGCCCCC      2150
AACATGGACT GCAACCAGTA AGTTCATTCA TTCTTTCTTA ACTCCAATTC      2200
AATTTATCCA TCACCTCGAC TTAAGCCTGA TTAAACTTAA CTTGTTCTTT      2250
GCATGCTTGC ACTATTGCAG GGTTACAGAG GAACTGAGAA TCTGAGAGCG      2300
TGAGGAATCG AGTTCATGTT GCATTTATCA TCAATCATCA TCGACTAGAT      2350
CAATAAATCG AGCAAAGCTT                                      2370
```

9. Sequence Description : SEQ ID NO. 8

SEQUENCE TYPE: nucleotide sequence
SEQUENCE LENGTH: 2407 bp

STRANDEDNESS: double stranded
TOPOLOGY: linear
MOLECULAR TYPE: genomic DNA

ORIGINAL SOURCE:
ORGANISM: Oryza sativa

FEATURES: - nt 1 to nt 2263 : sequence comprising anther-specific PE1 promoter
- nt 2181 to nt 2187 : TATA box
- nt 2211 : transcription initiation site determined by primer extension
- nt 2264 : ATG start of translation of E1 gene ; the sequence downstream from this position overlaps with the sequence of the cDNA of SEQ ID No. 5.

PROPERTIES: genomic DNA from Oryza sativa designated as GE1

```
TGATAGTGAC ATACTCACAT GCTTTGTCAA TTCAAGTATC AGTTCTTTTC      50
ATATTGATTT CTTAGTTGAT GAAAGTATAC ATATTTCTTG CCATCAATTC     100
TTTTAGTAGG TACATTTGGA CACTAGTGGT CAGGGTTGAA CTCTTAACTG     150
GAGTCTCATC TGATTTGCTT ATCTGAGACT GGGTTTGTGC AAATCCTGTC     200
ATGAGGCAAG GTGGACTGTC AGTCCATGAC ACTTTGCTAC TTCTATTAAG     250
TTCTCGAAAT CTTTTCCAGT GTATGTCCGT TCTCTTTCAA ATGAATTATT     300
TATATGTTCT GACAGCCTCG CGGTGTACAT TTCATTTAAC TTTTGTCTTC     350
ACAGGGCCTC TTGGTATTTT GTTGAGCAGA TTGGAATCAA CCTTCTTGTA     400
GAACTTCTTG ATGTCGTCGC TACCCTTTGC AACTAGATGG TCAACTTCTG     450
TCTTATATCT TTGGTACAAC ACTGGCAAAG TGTGCGCGCA CAAGAATCCT     500
GTGAAGTAAG AAATACAAAC TTGTCATTGT GAAAGTTTAG CTTTATATGA     550
TCTTGACTCT AAATTGTTTC TCCTCAGATC CTTCTGTGTG ATTGTTTTAT     600
TAAAATTTAA TATTTATCTG GAATACCTAC CAATATATAG TAGACTTGTC     650
AAGCTGCAAG AACTTCCAAT CGCCGACAAT ACCAATAGAG ATCCAACCAC     700
CTTAATATCA TAAACAATCT GATTGTTAGT CCAGAACTAT ATTGAGTAGT     750
GAACAACAAT AGCACATTAA CATTATGAGG ATTATTGGCT AACTCTGCAA     800
TTCAATATTC TGATGCGTCT AATCTGGTCA ATTTTAGCGC TCCAGAAAGA     850
ATTGCACAAT CCTTGGACAA TGTTGGCACT GGAACTGTTG CATGTTTTTA     900
CATCTCTTAT TAACGTAGCA AAGGAGTAGA TTATTATGTA CCAGGAGAAA     950
TCTCTTCAGA TCCTTTCCAC ATGCAATGTC GTAAAGAACA GATACAGTGT    1000
ACGTTAGTTT GTAATGGACG GTCAATGCCA TTTCTCTGAA GGCATGTTCA    1050
GAGATGATGA TTTCTGGGAT CCTTGGAGGG GCCCTGAAAT TCGGAAACAG    1100
TTAGTTGAGT TTTAGTACCT AATGTCTTGC GTTATACTAC GTGAAATGCC    1150
ATTTCTGTAA GCTGAGTTTT CTACCATCTC CACAGGAAAT AAAGCTAATA    1200
CCTGTCCAAG AGTGGTGCGG CATTTGACCA AATGAAGATC ACAAGCATGG    1250
CAAGAATGGC AATCTGGCAA AGGAGCGGAA TTATATTGTA TTCTACTACA    1300
TCGAACAGGA ACCATATCAA TGTTGCCCCA GCAAGGACCC CCGCAGATAA    1350
GTTCCTGTTC TTCCACAGCA GAATATCCGC AACTGCATAG CTCCCAACAA    1400
```

```
TGAAATCCAA AACCACATCG GCTCAGAGAG AAGTTATGAT AAAAGGCACT      1450
AATTCTGAAT AATTTCCTAG AAAGCGAATA ATAATAGCAC ACCTTGACCT      1500
CCACCAAGAA GCTTGTGGAT CGACTTGTGC CCATGAAATG GCATTCTGAC      1550
ATTCTGGTCA CTGTCAGAAT CTCTCGGAAA ATGAGGAGGC ATAGCTTCGT      1600
GTGTGTATGT GTGTGGGATA TTACGCTGCT AAAACTTTGT GTTTCTGATC      1650
GATCTGGTTA GAGAGCATCG TCTTTATAAG CACTTAAAAA TGGTAGTATA      1700
ATCTCTCAAG GAGCCTATAC TGCCAAGGAA AGGATAGCTT GGCCTGTGGG      1750
GATTGAGCCG TTGAAGGGAA CAAACGAATA CAGTTACCTT ACCAGATGTT      1800
TGCCACGACA TGGGCAACGT CATTGCTAGA CCAAGAAGGC AAGAAGCAAA      1850
GTTTAGCTGT CAAAAAAGAT ATGCTAGAGG CTTTCCAGAA TATGTTCTAT      1900
CTCAGCCAGA CCAATGGGGG CAAAATTTAC TACTATTTGC CATACATTAA      1950
CCACGTAAAA GTCCTACACT CAACCTAACT GTTGAACGGT CCTGTTCTGG      2000
CCAACGGTGA GAATGCACCT AATGGACGGG ACAACACTTC TTTCACCGTG      2050
CTACTGCTAC ATCCTGTAGA CGGTGGACGC GTGAGGTGCT TTCGCCATGA      2100
CCGTCCTTGG TTGTTGCAGT CACTTGCGCA CGCTTGCACC GTGACTCACC      2150
TGCCACATTG CCCCCGCCGT CGCCGGCGCC TACAAAAGCC ACACACGCAC      2200
GCCGGCCACG ATAACCCATC CTAGCATCCC GGTGTCCAGC AAGAGATCCA      2250
TCAAGCCGTC GCGATGACGA CGAGGCCTTC TGTTTTTTCC ACCGTTGTCG      2300
CGGCGATCGC CATCGCCGCG CTGCTGAGCA GCCTCCTCCT CCTGCAGGCT      2350
ACCCCGGCCG CGGCCAGCGC GAGGGCCTCG AAGAAGGCTT CGTGCGACCT      2400
GATGCAG                                                    2407
```

10. Sequence Description : SEQ ID NO. 9

SEQUENCE TYPE: nucleotide sequence
SEQUENCE LENGTH: 5620 bp

STRANDEDNESS: double stranded
TOPOLOGY: circular
MOLECULAR TYPE: plasmid DNA

FEATURES: - nt 1 to nt 395 : pUC18 derived sequence
- nt 396 to nt 802 : 3' regulatory sequence containing the polyadenylation site derived from the nopaline synthase gene from Agrobacterium T-DNA
- nt 803 to nt 1138 : coding sequence of the barnase gene
- nt 1139 to nt 1683 : sequence derived from tapetum-specific promoter of Nicotiana tabacum
- nt 1684 to nt 2516 : "35S3" promoter sequence derived from Cauliflower mosaic virus isolate CabbB-JI
- nt 2517 to nt 3068 : coding sequence of phosphinotricin acetyltransferase gene (bar)
- nt 3069 to nt 3356 : 3' regulatory sequence containing the polyadenylation site derived from Agrobacterium T-DNA nopaline synthase gene
- nt 3357 to nt 5620 : pUC18-derived sequence

PROPERTIES: plasmid DNA replicable in E. coli, designated as pVE108

```
TCGCGCGTTT CGGTGATGAC GGTGAAAACC TCTGACACAT GCAGCTCCCG      50
GAGACGGTCA CAGCTTGTCT GTAAGCGGAT GCCGGGAGCA GACAAGCCCG     100
TCAGGGCGCG TCAGCGGGTG TTGGCGGGTG TCGGGGCTGG CTTAACTATG     150
CGGCATCAGA GCAGATTGTA CTGAGAGTGC ACCATATGCG GTGTGAAATA     200
CCGCACAGAT GCGTAAGGAG AAAATACCGC ATCAGGCGCC ATTCGCCATT     250
CAGGCTGCGC AACTGTTGGG AAGGGCGATC GGTGCGGGCC TCTTCGCTAT     300
TACGCCAGCT GGCGAAAGGG GGATGTGCTG CAAGGCGATT AAGTTGGGTA     350
ACGCCAGGGT TTTCCCAGTC ACGACGTTGT AAAACGACGG CCAGTGAATT     400
CGAGCTCGGT ACCCGGGGAT CTTCCCGATC TAGTAACATA GATGACACCG     450
CGCGCGATAA TTTATCCTAG TTTGCGCGCT ATATTTTGTT TTCTATCGCG     500
TATTAAATGT ATAATTGCGG GACTCTAATC ATAAAAACCC ATCTCATAAA     550
TAACGTCATG CATTACATGT TAATTATTAC ATGCTTAACG TAATTCAACA     600
GAAATTATAT GATAATCATC GCAAGACCGG CAACAGGATT CAATCTTAAG     650
AAACTTTATT GCCAAATGTT TGAACGATCT GCTTCGGATC CTCTAGAGXX     700
XXCCGGAAAG TGAAATTGAC CGATCAGAGT TTGAAGAAAA ATTTATTACA     750
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CGTTATCTGA TTTTTGTAAA GGTCTGATAA TGGTCCGTTG TTTTGTAAAT     850
CAGCCAGTCG CTTGAGTAAA GAATCCGGTC TGAATTTCTG AAGCCTGATG     900
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TTGCCTTCCC TGTTTGAGAA GATGTCTCCG CCGATGCTTT TCCCCGGAGC    1000
GACGTCTGCA AGGTTCCCTT TTGATGCCAC CCAGCCGAGG GCTTGTGCTT    1050
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TCCGCAACCC CGTCAAACGT GTTGATAACC GGTACCATGG TAGCTAATTT    1150
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CACCACAAGG GCATATATAG AGCACAAGAC ATACACAACA ACTTGCAAAA 1250
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ACGATTCAAG GCTTGCTTCA TAAACCAAGG CAAGTAATAG AGATTGGAGT 1900
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GACGTCTAAG AAACCATTAT TATCATGACA TTAACCTATA AAAATAGGCG      5600
TATCACGAGG CCCTTTCGTC                                      5620
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CLAIMS

1. A stamen-specific plant promoter which can be isolated from genomic rice DNA, upstream of a gene having a DNA sequence which corresponds to T72 cDNA of SEQ ID no. 1, T23 cDNA of SEQ ID no. 2, T42 cDNA of SEQ ID no. 3, T155 cDNA of SEQ ID no. 4 or E1 cDNA, of SEQ ID no. 5, especially the T72 cDNA, particularly the promoter PT72 upstream from nucleotide 2846 of SEQ ID no. 6, the promoter PT42 upstream from nucleotide 1809 of SEQ ID no. 7, and the promoter PE1 upstream from nucleotide 2264 of SEQ ID no. 8.

2. A foreign, preferably chimaeric, DNA sequence suitable for transforming a plant, which comprises the promoter of claim 1 and a structural gene under the control of the promoter.

3. The foreign DNA sequence of claim 2 wherein the structural gene is a male-sterility DNA or a male fertility-restorer DNA.

4. The foreign DNA sequence of claim 2 or 3 which is a foreign chimaeric DNA sequence.

5. A plant cell or plant cell culture transformed with the foreign DNA sequence of anyone of claims 2-4.

6. A plant or its seeds consisting essentially of the plant cells of claim 5.

7. The genome of the plant of claim 6 containing the foreign DNA sequence of any one of claims 2-4.

8. A male-sterile plant of claim 6 in which the structural gene is a male-sterility DNA.

9. A fertility-restorer plant of claim 6 in which the structural gene is a male fertility-restorer DNA.

10. A male fertility-restored plant which is a hybrid of the male-sterile plant of claim 8 and the male fertility-restorer plant of claim 9 or the seeds of the male fertility-restored plant.

11. The plant of anyone of claims 6-10 which is a monocot.

INTERNATIONAL SEARCH REPORT

International Application No PCT/EP 92/00274

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C12N15/82; C12N15/29; A01H5/00; C12N5/10

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C12N ; A01H

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
A	WO,A,9 008 830 (ICI) 9 August 1990 see the whole document	.1-11
A	EP,A,0 344 029 (PLANT GENETIC SYSTEMS) 29 November 1989 see the whole document	1-11
A	Biological Abstracts, vol.87, ref.125906 & J.CELL SCI. vol. 92, no. 2, 1989, pages 217 - 230; RAGHAVAN, V.: 'Messenger RNAs and a cloned histone gene are differentially expressed during anther development in rice Oryza sativa L.' see the abstract	1-11

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° Special categories of cited documents :[10]

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
06 MAY 1992	20. 05. 92
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	MADDOX A.D.

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III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	J. CELL. BIOCHEM. SUPPL. vol. 15A, 1991, MEETING HELD JAN. 10-17 page 21; MARIANI, C., ET AL.: 'Genetic destruction of tapetal cells results in the production of male sterile plants' abstract A039	9,10
A	WO,A,9 008 828 (PALADIN HYBRIDS) 9 August 1990 see page 39, line 25 - page 48, line 31	9,10

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. EP 9200274 SA 55868

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 06/05/92
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9008830	09-08-90	AU-B- 621195	05-03-92
		AU-A- 4945690	24-08-90
		EP-A- 0455665	13-11-91
EP-A-0344029	29-11-89	AU-B- 621113	05-03-92
		AU-A- 3537189	24-11-89
		WO-A- 8910396	02-11-89
		JP-T- 2503988	22-11-90
WO-A-9008828	09-08-90	AU-A- 5037290	24-08-90
		EP-A- 0456706	21-11-91

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